Early Journal Content on JSTOR, Free to Anyone in the World

This article is one of nearly 500,000 scholarly works digitized and made freely available to everyone in the world by JSTOR.

Known as the Early Journal Content, this set of works include research articles, news, letters, and other writings published in more than 200 of the oldest leading academic journals. The works date from the mid-seventeenth to the early twentieth centuries.

We encourage people to read and share the Early Journal Content openly and to tell others that this resource exists. People may post this content online or redistribute in any way for non-commercial purposes.

Read more about Early Journal Content at http://about.jstor.org/participate-jstor/individuals/early-journal-content.

JSTOR is a digital library of academic journals, books, and primary source objects. JSTOR helps people discover, use, and build upon a wide range of content through a powerful research and teaching platform, and preserves this content for future generations. JSTOR is part of ITHAKA, a not-for-profit organization that also includes Ithaka S+R and Portico. For more information about JSTOR, please contact support@jstor.org.

FOOD ACCESSORY FACTORS (VITAMINS) IN BACTERIAL CULTURE WITH ESPECIAL REFERENCE TO HEMOPHILIC BACILLI I *

DAVID JOHN DAVIS

From the Departments of Pathology and Experimental Medicine of the University of Illinois, Chicago

The importance of food accessory substances or vitamins for the proper nourishment and growth of animals has come to be generally recognized. Through the work of Eykmaan it has been clearly shown that beriberi is caused by eating polished rice and can be cured or prevented by adding rice polishings to the food. As shown by Holst and others scurvy no doubt is a disease of a similar character due to the absence in the diet of certain substances found in fresh vegetables, fresh fruits, or fresh animal food. Several other both clinical and experimental diseases probably belong in this group. As far as the higher animals are concerned, the work of Hopkins, Funk, Osborne, and Mendel, McCollum, Moore, and Jackson, and others has now placed these deficiency diseases firmly on an experimental basis, and has shown clearly the necessity of accessory food bodies for proper development and for the maintenance of health. We may now regard these as essential food substances with proteins, carbohydrates, fats, and salts.

Evidence now indicates that the nutrition of the lower animal forms also may be dependent on somewhat similar specific substances. Loeb and Northrop[1] have pointed out that the fly, drosophila, requires yeast for its development. They succeeded in cultivating sterile flies and found that they grew and metamorphosed normally on sterilized baker's yeast and water. Mediums containing various salts and many proteins and amino-acids all failed to raise a single fly or pupa. They make the following concluding remarks:

> Yeast was then the only medium on which normal larvae could be raised with these sterile cultures of flies, and it seems to be the indispensable food for these insects. Yeast must therefore contain some substance required for their growth and this substance must be rather resistant to heat since yeast heated for one hour at 120 C. is an excellent culture medium. We tried to isolate this substance

---

* Received for publication June 6, 1917.

[1] Jour. Biol. Chem., 1916, 27, p. 309.

from the yeast. Yeast extracted with boiling alcohol for 48 hours or with cold alcohol for 10 days was no longer able to serve as food. A mixture of the extracted yeast and of the extractive was no longer adequate to raise the larvae indicating an alteration of the necessary substance by the alcohol. The presence of traces of alcohol in this mixture in itself was not harmful since these flies grow normally in the presence of 2 to 3 per cent. alcohol.

The necessary substance in yeast cannot be salts, or cane or grape sugar since non-sterile flies grow normally on Pasteur media, as Loeb's previous experiments have shown.

The addition of butter, nucleic acid, thymus or thyroid extract to the synthetic culture media mentioned in the table was also without effect. This fact, as well as the fact that the flies will not develop on sterile milk, shows that the necessary substance must be different from that needed for pigeons, rats, and other warm-blooded animals.

It is evident from this statement that the 'substance' in yeast on which the life of this fly depends, while its existence can scarcely be doubted, is, however, indefinite, and its properties and mode of action are quite obscure. It may, I think, quite properly be included in the group of food accessory substances.

The work of Bottomley has given almost equally important results in connection with the growth and development of the higher plants. Bottomley studied this question from the standpoint of soil fertility. He found that peat when inoculated with the nitrogen-fixing organism, Azotobacter, and placed in the air for a certain length of time and at a suitable temperature, contained not only certain food substances like potash, phosphates and organic substances, but also certain specific substances which had a powerful effect in inducing plant growth. These substances he was able to isolate from extracts of this 'bacterized' peat by methods quite like those used by Funk and others in obtaining animal vitamins from grains. When the crystalline bodies were added to soil in exceedingly small amounts, green plants planted therein grew and gained in weight much more rapidly than control plants. These substances Bottomley thinks are formed in the peat and also in ordinary soil under the influence of the nitrogen-fixing organisms, and to such bodies he has given the name 'auximones.' In order to detect the amount of these auximones in a given soil he uses a certain soil organism which readily produces a rich scum on an extract of soil containing them. He recommends the bacterized peat as an excellent fertilizer. This work has been confirmed by Jones[2] in this country who showed that heavy application of peat (10%) gave more than 100% increase of plant (radishes) by weight. The expense of preparation, however, may prevent its general application.

---

[2] Abstracts of Bacteriology, 1917, 1, p. 43.

The multiplicity of food accessory factors is generally admitted by investigators. The vitamins necessary to protect against beriberi are probably different from those necessary to protect against scurvy. Those concerned in the growth and development of the animal also seem to be multiple. Apparently certain ones of them are concerned with the growth, while others control the nutrition, and possibly still others determine the ability of the animal to bear young. Many investigators are accustomed to differentiate the power of foods to perform these various functions, this differentiation probably being based on the accessory food factors, or being somehow related to them.

In this respect the work of McCollum and his associates is important. Based on his many experiments in the feeding of animals he[3] divides the accessory food substances into 2 kinds: the fat soluble substance A and the water soluble substance B. The former is found among other places in butter fats, yolk of egg, meat, and impure lactose. The latter occurs especially in grains and generally in foodstuffs of vegetable and animal origin. Here he would include the vitamins of beriberi. He doubts the existence of more than 2 of these accessory food substances in any food, and makes the suggestion that scurvy is caused by intestinal derangement or stasis. As causes of faulty nutrition he enumerates in addition to these 2 food factors, a 3rd factor which concerns the inadequacy of the protein content because of an unsatisfactory yield of amino-acids, a 4th relating to the proper inorganic content of the food mixture, a 5th attributable to toxic substances in some natural foodstuffs like cotton seed and wheat products, and perhaps also a 6th factor concerned with the mechanical action of foods on the digestive tract, causing distention or feces of an unfavorable character.

Important points concerning the food accessory factors may be here briefly summarized. These bodies apparently comprise a large group of substances having certain properties in common but in many respects being quite different. A certain specificity seems to exist. The beriberi vitamin will not prevent scurvy, nor will certain substances that will cure or prevent scurvy have any effect on beriberi. They all seem to act in small amounts or in very high dilutions. Toward heat they are more or less tolerant, but in this respect considerable variation seems to exist. Some appear to withstand the boiling temperature for some time, others apparently do not. Some are water soluble and others are fat soluble. These substances may vary in their physiologic functions in that some seem to affect or control growth, whereas others are chiefly concerned with ordinary processes of waste and repair. Whether they are used or could serve as food alone is not known, but they seem to act as substances accessory to the other food constituents. Their mode or mechanism of action is not understood and their chemical nature is not known.

---

[3] Jour. Am. Med. Assn., 1917, 68, p. 1379.

A few years ago Dr. Moore and I undertook some experimental work in connection with the scurvy-like disease in guinea-pigs produced with milk, grains, and other foods. Since that time I have been interested in studying the question of the possible rôle that similar bodies might play in the growth of certain bacteria. The review of the rôle that these substances play in the nutrition of both the higher and lower animal forms, and also in higher plants would lead one to believe, a priori, that examples of the activity of similar substances might appear in bacterial nutrition.

This paper deals largely with the hemophilic bacteria. Pfeiffer[4] in 1892 described the influenza bacillus and in studying its properties made the discovery not only that blood was necessary for its continued growth, but also that hemoglobin was the essential constituent in the blood on which growth depended. This he did by obtaining the hemoglobin in pure crystalline form and adding it to ordinary mediums. These observations have been confirmed by many workers (Huber, Voges, Cantani, Ghon and Preyss and Luerssen), including myself. Serum and other constituents of the blood are not essential.

Pfeiffer did not make quantitative determinations as to the amount of hemoglobin necessary for growth. Several years ago while attempting to throw light on the mechanism of the action of hemoglobin in bacterial culture I[5] determined that hemoglobin was necessary in only very small amounts for the growth of hemophilic bacilli. In mediums containing as little as 1: 180,000 of hemoglobin the organism may continue to grow. Strains and mediums vary somewhat. In many of the experiments the dilution point of growth extinction was lower, being reached at dilutions 1: 10,000-1: 100,000. It was largely on this account that at that time I suggested that hemoglobin acted in the rôle of a catalytic agent in bacterial culture.

Czaplewski[6] also pointed out the small amount of hemoglobin that is necessary for the growth of the influenza bacilli. He used an amount of blood that hardly tinted the medium, and obtained with such dilutions even better growth than on the mediums containing large amounts of blood, such as Pfeiffer first used. I have also noted this fact that a small amount of blood is often preferable to larger amounts in growing these organisms. Czaplewski did not make definite quantitative determinations of the amount of hemoglobin necessary for growth. Ghon and Preyss[7] likewise noted that an extremely small amount of hemoglobin suffices for the growth of the influenza bacillus, stating that amounts of hemoglobin not demonstrable with the spectroscope are still sufficient for this purpose.

---

[4] Ztschr. f. Hyg. u. Infektionskrankh., 1893, 13, p. 357.

[5] Jour. Infect. Dis., 1907, 4, p. 73.

[6] Centralbl. f. Bakteriol., I, O., 1902, 32, p. 667.

Another point of importance is that hemoglobin, either in low or high concentration alone will not support the growth of these bacteria. This was pointed out by Ghon and Preyss[8] and also by Luerssen[9], who noted that hemoglobin in water agar is not a suitable medium. Even with the addition of peptone no growth will occur. By adding broth or milk to the hemoglobin growth will take place and continue indefinitely on transfer. I have recently gone over this work testing the growth of several strains of influenza bacilli in hemoglobin solutions from the dog, ox, sheep, human, cat, pig, and chicken. The solutions of hemoglobin used in this work were prepared by Dr. W. H. Welker of the Department of Physiology and Physiological Chemistry, University of Illinois, to whom I wish here to express my obligations. The hemoglobin crystals were added directly to water plus 0.5% sodium chlorid, to 2% water agar, to peptone water agar, and to ordinary plain agar. The bacilli continue to grow only in the last mentioned mediums. It would seem evident from these observations that the rôle of hemoglobin is not primarily that of a nutritive substance. I refer here only to the hemophilic bacteria, of course, since many other bacteria grow well in water solutions of pure hemoglobin or in all the above mentioned mediums.

It was noted by Pfeiffer that if blood-agar tubes were heated for 1 hour at 70 C. the hemophilic bacilli still readily grew on them, and he also found that hemoglobin coagulated by boiling would still support an appreciable growth though not a luxuriant one. From this observation he concluded that it was the iron content of the hemoglobin and not its oxygen-carrying power which in some way made possible the development of these organisms. He associated this phenomenon with the growth of other bacteria known as iron bacteria in water-containing iron. Ghon and Preyss[7] studied the relation of heat to hemoglobin and came to the conclusion that heat, even boiling temperature or above, did not destroy the power of the hemoglobin to stimulate growth. I have made similar observations and noted that heating even in the autoclave will not totally destroy this property of hemoglobin. I may point out that in order to obtain appreciable growth one must have in the mediums the particles of coagulated hemoglobin. If the brownish coagula are filtered out or are allowed to settle out the medium will yield little or no growth. When such a solution of coagulated hemoglobin is smeared on agar and the medium inoculated the growth is seen to occur immediately about the coagulated particles, but not elsewhere. With such solutions of coagulated hemoglobin, while growth does occur, it is as a rule not as profuse as with the unheated hemoglobin, unless another organism is grown in symbiosis with the hemophilic bacillus. This symbiotic relationship I shall discuss presently. We must conclude, therefore, that coagulation by heat and heating even above the boiling point does not entirely destroy the power of hemoglobin to support the growth of these bacteria.

---

[7] Centralbl. f. Bakteriol., 1902, 32, p. 96.

[8] Ibid., 35, p. 531.

[9] Ibid., p. 434.

To sum up then the characteristics of hemoglobin in relation to the growth of hemophilic bacteria we may state that (1) hemoglobin is essential for their growth; (2) it is sufficient in high dilution; (3) alone, it will not support growth, other proteins being necessary for continued development, and (4) it does not lose its power of supporting growth, at least not entirely, through prolonged heating at the boiling point or even higher. On analyzing these points one is struck by the close correspondence between them and the properties and mode of action, as far as is known, of the food accessory factors in the higher forms of life. Further discussion of these points will appear.

In addition to the hemoglobin factor, another factor enters in the growth of hemophilic bacilli. It was noted first by Grassberger[10] that when these bacteria were grown on hemoglobin mediums, if mixed with other organisms, the growth was decidedly more abundant. He notes this phenomenon especially about staphylococcus colonies, but other organisms produce the same effect. It was this observation which first gave rise to the common practice of inoculating the entire surface of a slant with influenza bacilli and then making a staphylococcus streak through the center. If on blood plates sown with influenza bacilli other organisms are grown, large colonies of the former will appear about the latter, producing a striking appearance and suggesting a satellite relation. This influence extends to a distance of 2-4 m. or more about the foreign colony. There seems to be little difference as to what organism is used as a companion for the influenza bacillus. I have tested many different organisms in this regard but they all give quite uniform results. Staphylococci, streptococci, both hemolytic and nonhemolytic, pneumococci, meningococci, diphtheria bacilli, various chromogens, blastomycetes, sporothricha, yeasts, and many others all show this phenomenon with influenza bacilli. It does not depend on hemolysis since both hemolyzers and nonhemolyzers manifest it. Nor is it dependent on reaction since both acid- and alkali-producers show it.

---

[10] Ztschr. f. Hyg. u. Infektionskrankh., 1897, 25, p. 453.

I have made a thorough search for substances which might behave like these bacterial colonies, using for this purpose a hemoglobin-agar plate rather thickly sown with influenza bacilli. In addition to bacteria, pieces of fresh animal tissue show this phenomenon. Kidney, liver, spleen, brain, myocardium, testicle, lung, and muscle of the rabbit and guinea-pig were tested, and all yielded quite uniform results. A drop of fresh blood on the surface of the blood-agar plates shows little or no such activity. This stimulation of growth does not depend on the hemoglobin in the tissues. A small piece of the inside of a potato or carrot placed on the surface also shows this symbiotic effect exactly like the animal tissues. It is needless to state that all such tissues must be obtained under sterile precautions, since other bacteria would produce the same result. If one heats the pieces of animal or plant tissues to 60 C. for 30 minutes they still manifest this stimulating action. When heated to boiling, however, for 15 minutes the tissues, both plant and animal, lose this power. Killed bacteria also lose to some extent but not entirely their influence on such hemoglobin plates. The animal tissues, since they contain hemoglobin, when placed on plain agar plates sown with influenza bacilli, support growth of these bacilli as one would expect. However, carrot or potato tissue, unheated or heated, when added to plain agar, yield no growth whatever. Animal substances free from hemoglobin, like spermatic fluid, yolk or white of egg, spinal fluid, ascites fluid, etc., will not support growth consistently, but on sterile sputum and bile mediums I have obtained definite growth for 1 or 2 generations. In mixed cultures also influenza bacilli may grow on nonhemoglobin mediums for some time. Neisser[11] claimed that he grew them in mixed cultures with B. xerosis for 20 generations in plain agar. I have grown them with B. diphtheriae on plain agar through 6 generations. Ordinarily, however, they soon disappear from the mixed cultures after a few transfers.

Ghon and Preyss have explained this growth on plain mediums with other organisms, no doubt correctly, by showing that plain mediums often may contain appreciable quantities of hematin, which, when associated with other bacteria, will support the growth of hemophilic bacilli. Hematin, however, alone, unlike hemoglobin, added to mediums will not permit their growth; only when it is mixed with bacteria will it exercise its power in this respect. These investigators noted also that either living or dead bacteria, killed by heat or otherwise, when associated with hematin would cause abundant growth of influenza bacilli. Either substance alone would not do this. The hematin which they used was prepared by digesting acidified hemoglobin with pepsin for several weeks, and the deposit purified by filtration, washing, and extraction with ether. This solution keeps for a long time. A suspension of bacteria may also be prepared and plain agar to which the bacteria and hematin are added, they claim, makes a very satisfactory medium for continued cultivation of the organisms. I have tested hematin agar with and without bacteria and can confirm these results. Hematin alone in mediums will not yield growth; with bacteria good growth takes place.

It seems then that in plain agar hemoglobin alone will yield growth, but when it is associated with bacteria or tissues, either plant or animal, especially if alive, there occurs what we may designate an optimum growth. Plant and animal tissues and bacteria by themselves will not sustain growth. It appears, therefore, that there is some factor or factors in the latter tissues which somehow act with the hemoglobin to favor growth. Hematin alone will not sustain growth, but associated with bacteria it will do so.

It is interesting to note here the close correspondence that seems to exist between these 2 growth factors in bacterial culture, namely, hemoglobin and the tissue factor, and the 2 substances detected by McCollum and his associates in their diet experiments with animals. McCollum differentiated, as already stated, 2 unidentified dietary factors, a fat soluble substance A and a water soluble substance B, and describes them thus:

---

[11] Deutsch. med. Wchnschr., 1903, 29, p. 462.

> One (substance B) is soluble in water and alcohol and apparently never associated with the lipoids, when these are isolated from natural food stuffs, the other (substance A) soluble in fats. The latter is extracted from milk, egg yolk, kidney and probably other animal tissues by ether but is not removed by this solvent from either the seeds or the leaves of plants. The former is universally present in foodstuffs of vegetable and animal origin but is absent or nearly so from crystalline sugar, starch, and fats, and is present in but small amounts in polished rice and probably on those foods derived from the endosperm of seeds by milling processes. Where the reaction of the medium is alkaline, strong heating of foods probably leads to its destruction.

It is evident, however, that the substances A and B of McCollum are different in many respects from the substances necessary for bacterial culture, but in general it may be said they are analogous or may indeed be placed together in groups with certain common properties. For example, hemoglobin is a water and alcohol soluble substance and may possibly be grouped with the B substance of McCollum. The other factor in influenzal culture is closely associated with other bacteria and living or fresh animal and plant tissue. The question may be asked whether this factor or substance might possess in general the properties of substance A, which according to McCollum is found in the kidney and probably other tissues, in milk, eggs, etc.

The properties of the 2d substance in influenzal culture as yet have not been studied very carefully. This is being carried out at present along lines which may permit us to compare them with the other accessory substances in diets of higher animals. Ghon and Preyss found that ash of staphylococci would not behave as did the dead or living bacteria with hematin. The substance, therefore, is organic. Whether it resides in the bacterial body or results from the action of the bacteria or tissues on the mediums is not known. It may be doubted, too, whether one and the same substance is responsible for the symbiotic effect of bacteria or tissue in hemoglobin mediums and the satellite phenomenon observed on hematin agar. There seems to be some difference in the resistance to heat of the substances concerned in this phenomenon. This point should be studied further. So far as the 2d substance is concerned then we know perhaps even less about its nature and mode of action than we do about the vitamins or the substances of McCollum, though its existence and influence can not be doubted.

Assuming that hemoglobin behaves as a food accessory substance in bacterial nutrition it is possible that through a study of its action we may be able to throw light on the mechanism of the activity of food accessory substances in general. We know much more about the chemistry and the function of hemoglobin than we do about vitamins. However, at the present moment, we cannot say with any certainty how hemoglobin acts in supporting the growth of hemophils. In the body the chief function of hemoglobin is to carry oxygen, but it evidently does not serve in this capacity for bacteria, since coagulating and heating does not destroy its usefulness for the latter, but does destroy its oxygen-carrying function. Furthermore, hemocyanin,[5] a copper-containing substances in the blood of many lower animals, performs the same function as an oxygen-carrier as hemoglobin but does not support the growth of hemophils. It is not poisonous to them for when hemoglobin is added to the mediums containing hemocyanin the organisms proceed to develop normally. Pfeiffer thought the iron of the hemoglobin was the essential substance, and this is the opinion of Luerssen and Ghon and Preyss. The latter, who made the most careful study of this point, believe that the iron must exist in an organic form readily obtainable and that other bacteria by their reducing action make the iron of the hemoglobin and hematin available for the hemophilic bacteria. It should be stated that various iron salts and organic preparations of iron will not serve in place of hemoglobin. Hematogen, ferratin, and many other preparations have been tested with negative results. Cantani and Ghon and Preyss found that bile from certain persons, but not from all, would behave much as hematin behaves. Bile pigments and salts gave negative results. Since bile from different persons varies in its iron-content these results may depend on the iron-content, though this point was not determined by them. Ghon and Preyss after trying many iron preparations succeeded in getting some growth of influenza bacteria with foreign organism by adding to ordinary mediums an iron preparation made by dissolving iron hydroxid in hydrocyanic acid, and sterilizing by boiling. They state that when this was added in small amounts to agar, with foreign organisms, growth of the hemophils regularly occurred about the latter, though the colonies were indeed small. The point should be emphasized, however, that hemoglobin is the only substance known which alone supports the growth of these bacteria. All other iron-containing substances, such as hematin, require the aid of foreign cells.

Recently Dorothy Lloyd[12] in England has called attention to the rôle of vitamins in the growth of the meningococcus. She concludes from the study that the primary cultivation of the meningococcus in vitro is only possible in the presence of certain accessory growth factors present in blood, serum, milk, and other animal fluids, and probably present also in vegetable tissues. These accessory factors are bodies which are moderately heat-stable, and are soluble in water and alcohol. They are rapidly adsorbed from solution by filter paper, but do not appear to be adsorbed by glass wool. On account of an inverse relationship between the amount of amino-acid present in the medium and the amount of vitamins required to stimulate the growth of laboratory strains it is suggested that the action of the accessory growth factors is to increase the reaction velocity of the proteolytic metabolism of the meningococcus. The meningococcus after isolation from the body gradually develops a change in its metabolism of such a nature that it becomes increasingly independent of a vitamin supply on the medium. Old laboratory strains need no additional vitamin supply if the medium contains an abundant supply of free amino-acids. She was able to obtain the stimulating factors from fresh bood by extracting it with 80% alcohol. Evidently the substance with which she is dealing is closely related to the accessory factors in the diet of animals.

The question naturally arises as to the rôle of such substances in the growth of other varieties of bacteria and especially the pathogenic forms in the body. We are familiar with the important rôle that blood, body fluids, fresh animal tissue, and undenatured proteins play in the cultivation of many varieties. The suggestion seems reasonable that 1 of the determining factors for such growth may be the vitamin-content of the tissues for specific kinds of organisms. This subject, therefore, at once becomes related to problems of virulence, susceptibility, and immunity.

SUMMARY

Food accessory factors are now recognized for higher animals, for certain lower forms, for the higher plants, and for bacteria.

These substances are necessary for proper nutrititon in addition to proteins, fats, carbohydrates, and salts.

They act in very small amounts and probably by themselves, and, at least in case of certain bacteria, are not able to support growth. They are relatively resistant to heat.

---

[12] Jour. Path. and Bacteriol., 1916, 21, p. 113.

For the growth of hemophilic bacteria pure hemoglobin is 1 factor and a 2d factor resides in foreign bacteria and fresh animal and plant tissue. To obtain maximum development both factors are necessary. Hematin alone does not support growth but does so in conjunction with other bacteria, living or dead. The similarity of the action of these 2 factors to the action of the fat soluble substance A and the water soluble substance B of McCollum is pointed out.

The mechanism of the action of such substances is not clear. The availability of iron in the hemoglobin may be the determining factor in the growth of hemophils. The 2d factor or substance may act by rendering this element more available.

The multiplicity of food accessory factors is recognized by most investigators. Their mode of activity may be as varied. Their action in connection with hemophilic bacteria seems to center around the metabolism of iron.

The suggestion may be made, in conclusion, that the activity of these substances in animals and higher plants may concern or somehow control the metabolism of certain elements like iron, phosphorus, calcium, or iodin, as well as possibly the protein metabolism.